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IBScientific Journal of Science

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**The androgen receptor and its role in
the progression of prostate cancer**

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The androgen receptor and its role in progression of prostate cancer from androgen dependence to androgen independence.

Greg N. BROOKE and Charlotte L. BEVAN

EDITORIAL

IBScientific, In Search for New Roots

Since its launch, the initiative of IBScientific has primarily aimed to promote scientific publishing and communication with a profound vision to establish a backbone for Algerian science back home and abroad. With the electronic publishing of IBScientific Magazine and IBScientific Journal of Science, the seed of IBScientific produced its offshoots, which went on to stand strong and firm through its striving continuity. Of course, it is not unreasonable to say that the seed of IBScientific has also made it to flowering. Indeed, holding the 1st IBScientific Annual Conference is a good point of illustration.

With this in mind, it is now important to think about the roots of the IBScientific plant; after all it is the roots that keep the plant nourished. Up to now, the roots of IBScientific have been no more than a commitment and a determination of a few young people who strongly believe in the idea, mission, and vision. It is now time to suggest that IBScientific may need yet another set of roots to keep pace with the growing challenges, especially in relation to improving science back home in Algeria. Such an approach may involve the establishment of a home-based Algerian Academy of Science which would initially act as hub that links the Algerian scientific community with that

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present abroad. Alongside with IBScientific, the academy can contribute to “investigating, examining, experimenting and reporting upon any subject of science or art”. In the long term, the academy may facilitate and regulate research funding, acknowledge distinguished scientists, and provide consultancy to policy makers on relevant issues. Most importantly, the academy would allow the appropriate use of science for the general welfare,

and keep track with the growing roles that science and technology play in public life. Undoubtedly, these entire objectives are part of the IBScientific vision, so it may be The Algerian Academy of Science starting.

Abderrahmane KAIDI
IBScientific Board Member

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The Androgen Receptor: Good Cop vs. Bad Cop in Arresting Prostate Cancer?

Preview by: **Iain J. McEwan**

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Prostate cancer (PCa) originates in the epithelial compartment of the prostate gland and is fast becoming the leading cause of cancer-related death in Western men; over 30,000 new cases are diagnosed in the UK each year (6). The androgenic steroid hormones, testosterone and the 5α -reduced metabolite dehydrotestosterone play important roles in prostate development, growth and function and act through the intracellular androgen receptor (AR), a member of the nuclear receptor superfamily (Figure 1). The receptor protein consists of a C-terminal ligand-binding domain (LBD), that folds to form a three layer α -helical sandwich. This is connected via a hinge domain to the DNA binding (DBD), which has a globular fold made-up of two perpendicular α -helices. The structurally distinct large N-terminal domain has a flexible conformation with a propensity to form α -helix, and is important for multiple-protein-protein interactions and transcription regulation (5). PCa treatment primarily involves targeting the androgen-signalling pathway by blocking production of testicular androgens and inhibiting AR function (3). While initially such treatments are successful in manag-

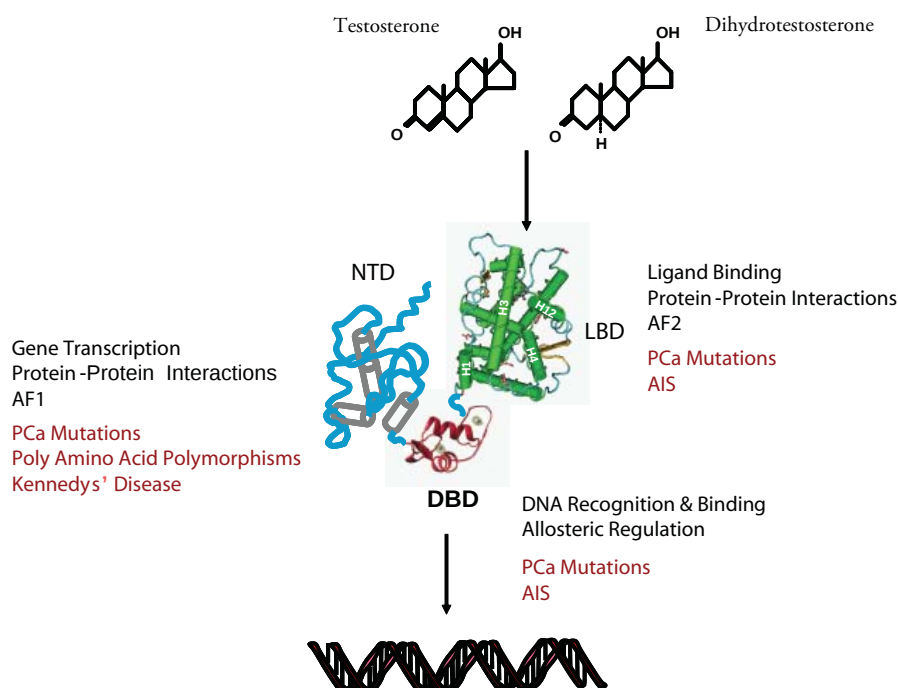


Figure 1: Structural and Functional Domains of the Androgen Receptor. The AR has a characteristic domain organisation consisting of a ligand-binding domain (LBD), hinge, DNA-binding domain (DBD) and N-terminal domain (NTD). High-resolution structures are available for the individual LBD, with different agonists and antagonists bound, and DBD only, while the NTD is structurally flexible with regions of expected α -helix. Point mutations have been described in clinical samples of prostate cancer (PCa) within the LBD, DBD and NTD generally, but not exclusively, after androgen-ablation therapy. Mutations have also been described in the LBD and DBD that lead to a disruption of male differentiation: androgen insensitivity syndrome (AIS) and polymorphisms in the large poly-glutamine repeat, in the NTD, results in a late-on-set neurodegenerative conditions termed Kennedy's disease. AF1 and 2, activation function 1 and 2.

ing the disease, the period of remission is variable and inevitably the tumour escapes this androgen ablation therapy and progresses to a hormone-independent state with concomitant poor prognosis. However, the hormone resistant tumours still retain a functional AR and progression may be associated with amplification of the receptor gene, mutations of the receptor protein leading to promiscuous activation by non-androgenic steroids, alterations in co-factor levels and/or cross talk with growth factor / cytokine signalling pathways (see 3, 1). In the Review article on page 48, Brooke and Bevan (1) comprehensively summarises our current understanding of AR signalling in hormone-responsive and hormone-refractory PCa: including, AR structure-function, current therapies in PCa and the mechanisms leading to androgen-independent disease.

Importantly, deregulation of the AR signalling pathway, as outlined above, has been shown to result in anti-androgens acting as agonists to switch on the receptor (2, 7). Thus, the main take home message is that the AR 'remains a valid therapeutic target' and that a better understanding of the AR signalling in the healthy and diseased prostate will be important for the development of novel therapeutic strategies, especially in the late stages of hormone-refractory prostate cancer, where currently patient prognosis is poor.

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INTERVIEW

Dr. Elias A. ZERHOUNI

Director of the National Institutes of Health (NIH)
United States of America

Conducted for IBS JoS by:
Dr. M. Tayebi

Elias A. Zerhouni, M.D., is the 15th and current Director of the National Institutes of Health (NIH), leads the United States' medical research agency and oversees the NIH's 27 Institutes and Centers with a budget this year of \$28.6 billion. His accomplishments at the NIH have included: overseeing the completion of the doubling of the NIH budget; initiating the NIH Roadmap for Medical Research; establishing an NIH-wide research initiative to address the obesity epidemic; supporting the NIH Neuroscience Blueprint; Supporting the reduction of health disparities and barriers to opportunity for minority individuals; and ensuring public access to NIH-funded research results.

Prior to joining the NIH, Dr. Zerhouni served as executive vice-dean of Johns Hopkins University School of Medicine, chair of the Russell H. Morgan department of radiology and radiological science, and Martin Donner professor of radiology, and professor of biomedical engineering. Before that, he was vice dean for research at Johns Hopkins.

A resident of Baltimore, Maryland, Dr. Zerhouni is actually North African, having been born in Nedroma, Algeria. He came to the United States at age 24, having earned his medical degree at the University of Algiers



Dr. Elias A. Zerhouni, Director of the National Institutes of Health since May, 2002

School of Medicine in 1975.

After completing his residency in diagnostic radiology at Johns Hopkins in 1978 as chief resident, he served as assistant professor in 1979 and associate professor in 1985. Between 1981 and 1985 he was in the department of radiology at Eastern Virginia Medical School and its affiliated DePaul Hospital. In 1985, Dr. Zerhouni returned to Johns Hopkins where he was appointed director of the MRI division, and then was appointed full professor in 1992, becoming the chairman of the radiology department in January 1996. He has been ranked multiple times in "Best Doctors in America."

Since 2000, he has been a member of the National Academy of Sciences' Institute of Medicine. He had served on the National Cancer Institute's Board of Scientific Advisors from

1998 to 2002. He has won several awards for his research including a Gold Medal from the American Roentgen Ray Society for CT research and two Paul Lauterbur Awards for MRI research. His research in imaging led to advances in Computed tomography (CAT scanning) and Magnetic Resonance Imaging (MRI) that resulted in 157 peer reviewed publications and 8 patents.

Dr. Zerhouni received the honorary title Doctor Emeritus from the University of Algiers in 2005.

Dr. Tayebi: Thank you for accepting this interview. It is much appreciated.

I'm grateful for the opportunity to communicate with your readers.

Dr. Tayebi: What attracted you to the NIH?

Two things, really: the opportunity to determine the direction of the country's leading medical research agency and the satisfaction of working with colleagues from all over the world to improve health.

Dr. Tayebi: You have visited Algeria on few occasions. Some of the visits were high profile. Could you provide some background for the visits?

Naturally, I've welcomed opportunities to encourage the development of robust, evidence-based healthcare in the country of my birth. I considered it a great honour to be awarded an honorary degree from the institution where I spent the formative years of my training. I hope that, in accepting that honour, I was also able to provide some inspiration to the students who follow. When I returned almost a year later, this past January, I had two goals: The first was to witness the signing of a U.S.-Algeria Science and Technology Agreement. In addition to providing the legal framework for collaboration in all areas of science, I see this document as a milepost declaring that both governments recognize the leading role of science in societies and, most importantly, acknowledge the fact that scientific development is enhanced when countries cooperate. My second goal was to visit as many research and healthcare facilities as I could in the short amount of time available, to gain a better sense of what's happening on the ground. The better we know each other, the better we can work together. I felt strongly about this, so I took with me a group of NIH staff from a variety of institutes who had already been working to establish ties with scientists throughout North Africa.

Dr. Tayebi: How do you rate the outcome of the exchanges you had?

We learned a lot, but we knew the real work would begin after the visit. And that is exactly what has happened. E-mail communications are flying back and forth; a few Algerian scientists have attended workshops, with more to come; numerous small planning groups, formal and informal, are thinking and talking about what we heard from our Algerian colleagues in terms of their training needs and how we might try helping each other meet those needs. It can appear to be a slow and cumbersome process, with each step requiring a lot of commitment and work from both sides, but we are committed to continue working at it.

Dr. Tayebi: What are the main obstacles to achieve the agreed goals with the Algerian authorities?

Of course there are shared challenges, but I don't think in terms of obstacles. I think in terms of opportunities. My experience out of my meetings with senior government officials is that the political will is there to confront the challenges, as best we can, as they arise. But if we're not working together to pursue the opportunities, we won't even get that far. As with any new undertaking, the challenge of communication is important to overcome. To the extent that US and Algerian colleagues can understand shared priorities, as well as

mechanisms to support cooperation, the partnerships will flourish.

Dr. Tayebi: You have participated in the founding meeting for Arab Expatriate Scientists in Doha, Qatar. Some Arab scientists perceive this type of initiative in the Arab world as another 'white elephant'? What is your view on that? How could this initiative be expanded to other areas in the region?

White elephants are revered in some parts of the world. I think the point is that there will be no "one size fits all" solution. A variety of approaches will be needed to meet the educational and research needs of the region. If strategies can be identified that can, in some contexts, provide high calibre training and research opportunities, while providing an opportunity to constructively address the question of the potential role of expatriate scientists, I think that's laudable. The key is to identify, recruit and retain the very best scientists and give them a very supportive environment.

Dr. Tayebi: As far as Algeria is concerned, some suggest that we have all necessary 'ingredients' available to achieve high profile research both nationally and globally. Instead, Algerian science is not able to take-off. What is the problem?

I'm not sure I can agree with either statement. Perhaps a cooking analogy is not the best one for thinking about the whole research enterprise. It's a pretty heady statement to suggest that any one country has everything it takes. It's a process and one which is not easily mapped, particularly in the context of a country that has endured (but overcome) a long period of internal conflict. Some patience is called for, to give time for the talented and very hard-working Algerian scientists to gain momentum. To the extent that governments support environments that support the best and the brightest in science, the enterprise will grow. Without a long term commitment and respect for science by the people and at the highest levels of government, it is difficult to become a strong Nation in science.

Dr. Tayebi: Are NIH funds available to Algerian institutions?

Yes, on a competitive basis. NIH accepts applications from anywhere in the world. Scientists are invited to apply either as partners with U.S. colleagues or as independent investigators. Our experience is that foreign scientists are often more successful if they have collaborators who, for reasons of language or experience in the U.S., are already familiar with the NIH funding process.

Dr. Tayebi: Could the NIH provide any figures of Algerian institutions funded by the NIH?

There are no grant awards at this time directly involving Algerian institutions. However, there are a small number of Algerians working and training in NIH laboratories and there are certainly Algerian scientists engaged in research that is funded by the NIH at institutions in Europe and the United States. During my trip, we used every opportunity to discuss the NIH Visiting Program as a means of matching bright young minds from Algeria to the NIH intramural program. One of the obstacles is that few Algerian scientists can communicate well enough in English, which is the international language for science and technology.

Dr. Tayebi: Is there any barrier in transferring the funds to countries like Algeria? Could you provide an example where the NIH is actively funding projects in developing countries?

Dr E Zerhouni: There are no barriers to transferring grant funding to countries like Algeria. The Institutes and Centers of the NIH make awards involving institutions all over the world, including Southeast Asia, Latin America and Sub-Saharan Africa. One NIH Center, the Fogarty International Center, has focused on research and research training programs in low and middle income countries.

Dr. Tayebi: Can Algerian scientists from private institutions apply for such funds?

In general, yes. If an application is submitted in response to a call for proposals, the question of institutional eligibility is addressed in detail in the announcement.

Dr. Tayebi: Can Algerian Scientists and students attend US laboratories and institutions through an NIH initiative?

The NIH Visiting Program is an important element in our strategy to train the next generation of health researchers. Each year, the Visiting Program hosts about 2700 foreign scientists on the NIH campus in Bethesda, Maryland or at other NIH intramural laboratories with the U.S. Participation in the Visiting Program is based on the scientific match and interest between the prospective participants – primarily post-doctoral fellows - and the laboratory chiefs. It's up to the scientists involved to find that match. There are a few other programs through which foreign scientists receive training as part of specific NIH training and research grants at U.S. universities. The NIH website is the tool to begin exploring funding opportunities.

Dr. Tayebi: How has science changed over your career? How much further is there to go?

I feel privileged to have witnessed during my career what I consider to be the landmark for medical research: the completion of the human genome project in 2003. With that and our understanding of the molecular basis of disease, we can move from curing to pre-empting disease. Some say the 20th century belonged to physics, the 21st to biology. That's true in a certain sense, but I think the most exciting challenge will be to link knowledge about genes, behaviour, nutrition, infectious agents, environment and social and cultural factors to better pre-empt, prevent and treat disease. We'll need to find new ways to build relationships, together with the social scientists, mathematicians and physicists. The road ahead is boundless.

Dr. Tayebi: How did your publications contribute to your achievements? In your experience, how could publication contribute to science development in developing and developed countries?

Publications can be many things: an achievement, a starting point or an end point. They can signify how far we've come or how far we have to go. They are tools, not only to disseminate information or provide answers, but to stimulate questions. The best publications make their greatest contribution by inviting, sometimes provoking, further discourse, investigation and exchange of ideas.

Dr. Tayebi: Some would argue that we first promote research then publication, or should the two be promoted equally?

I think if one logically follows through what I've already said, there is no "first this, then that." The key is motivation and a supporting environment that respects and encourages high-quality science with no compromises on excellence and proven merit.

Dr. Tayebi: Giving the situation of publishing in Algeria, How do you think we should promote the importance of publishing among Algerian scientists? How do we engage with the wider population?

Again, this question cannot be separated from the issue of high calibre research. Lancet recently published a Brief on medical journals in the Middle East [vol. 367, March 25, 2006]. It suggests that the journals in the region receive very few submissions from other parts of the world or even from neighbouring countries. And the journals have to compete for manuscripts among the limited number available from the region that deal with clinical research, for example. Other challenges exist, as well, I know, but recognition of the importance of publishing and the quality of research will go hand-in-hand. Given the calibre of the human resources and the political will I've seen in the region, I'm convinced both will advance.

Dr. Tayebi: How would you describe the importance of collaboration in research?

Vital. Without collaboration we can't even begin to think about the multi-disciplinary research teams we know will drive future advances in health research. And the critical role of international collaborations cannot be overstated. New knowledge about diabetes, depression and AIDS are just a few among the many advances made in the past two years through international collaborations. This is reflected in journals: By 2001, almost one quarter of all U.S. scientific articles had at least one non-U.S. co-author, compared to 10% in 1988. [Science and Engineering indicators, 2004, National Science Foundation]

Dr. Tayebi: What kind of collaboration will be most beneficial to science in Algeria: training, peer-to-peer or materials based?

Having had the privilege of knowing very distinguished, seasoned Algerian scientists as well as individuals just beginning their careers, I would say we are all in need of all kinds of collaborations. However, my NIH colleagues who accompanied me on my January trip to Algiers - and spent an additional day there - tell me, unsurprisingly, that the Algerians brought up the need for training repeatedly. Interestingly, training opportunities in Algeria was the preference. Also, Algerian scientists have to become proficient in English, which can open many doors beyond the French-speaking world.

Dr. Tayebi: What are the best ways of promoting collaboration with Algeria? How can we attract and convince scientists from developed world to work/collaborate with Algerian institutions?

The emphasis need not be on attracting or convincing scientists to collaborate with Algerian institutions or scientists. Productive collaborations will not come about because it's Algeria or Sweden or

Japan or any other particular country. Good science (good scientists) and new or unique opportunities for research will be the attraction. But it is also the responsibility of Algerian scientists to make their work known and to remain as informed as possible about the most recent advances related to their field, in order to be in a position to really engage their international colleagues. If Algerian scientists can bring unique contributions, collaborations will grow!

Dr. Tayebi: Publishing is meant to be the main driver for attracting international collaborators. Any thoughts?

Yes, we have made that point here a number of times – it certainly is one of the drivers for developing research collaborations.

Dr. Tayebi: Should Algeria promote basic over applied research?

There is no stock answer for this question. At the NIH, the balance has remained fairly constant over the last decade, with a range between 10-15% more basic research than applied. When the priority-setting is driven primarily by the scientific community, a balance will be established that is likely to reflect Algerian needs. The key is first to promote science and scientists. The rest will follow because there is a pool of untapped potential and talent in Algeria.

Dr. Tayebi: Some suggest that Algeria should concentrate only on research that is needed locally. Opponents suggest instead that R&D should have no frontiers. What is your view?

Specific to health-related research, in a recent publication on global health, my colleagues suggested that “all health care is national, and all health research is global.” [Disease Control Priorities in Developing Countries, 2nd edition, p 104] Think about the fact that, in all fields R&D is, after all, one of the drivers of globalisation. Nonetheless, it is important for scientists to address locally important problems if they are to be supported by their society.

Dr. Tayebi: How can we convince the political establishment about the importance of promoting innovation/entrepreneurship as an engine for economical development?

The political establishment is convinced. As in everything, it's a matter of establishing priorities to find a workable balance for what can appear to be competing interests, find the right leaders and define operating principles that drive towards excellence and merit, without convenient compromises. Clearly, this means being open to advice, constructive criticism, good collaborations between Algerian scien-

tists and internationally.

Dr. Tayebi: The International Brain Research Organization (IBRO) has recently organised the first international neuroscience conference in Algiers, Algeria. Its regional director, Professor Raj N. Kalaria put emphasize on using the English language as medium for promoting and communicating research in Algeria. He also mentioned that it was of paramount importance to equip Algerian scientists with an acceptable level of English to stand a better chance of access to international funds and collaborations. Do you share his view?

Absolutely. And I have never met an Algerian scientist who disagreed.

Dr. Tayebi: As an American-Algerian, and having achieved so much, what is your message to the Algerian youth and to the Algerian scientific community worldwide?

It's important to reflect on what we have, collectively, to be thankful for – Algeria is a country at the crossroads of the Middle East, Europe and Africa. Ideally, our own cultural environment could encourage us to be curious about other peoples and other cultures. We've also had the opportunity to learn to overcome adversity, with tenacity and energy. Curiosity, tenacity, energy – all attributes required by any decent scientist.

Dr. Tayebi: Finally, what do you think about Zizou? Some say that his 'head-butting' was typically Algerian. Was it?

He is a star. I watched all his games. His response to insults is typical of the way we grew up but I think he should have controlled himself and kept his majesty.

Dr. Tayebi: I read that you are still a great fan of Algerian cuisine. Are you a 'chorba' or a 'couscous' type of person?

Both. And 'mechoui' too!

Dr. Tayebi: Dr Zerhouni; on behalf of IBScientific board I would like to extend my warmest thanks to you and also for giving me this great opportunity to communicate your thoughts and experience to IBScientific audience. Thank You!

Thank you.!

The androgen receptor and its role in progression of prostate cancer from androgen dependence to androgen independence

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ABSTRACT

Prostate Cancer is a leading cause of cancer-related death in Western men. Growth of prostate tumours is dependent upon the androgen signalling pathway and therapies that block this signalling axis have a high success rate in reducing tumour growth. Such therapies, however, invariably eventually fail and the tumour progresses in the androgen-depleted environment. The androgen receptor is still expressed in the majority of such androgen independent cancers and is believed to be still required for growth. This review summarises the current knowledge on the structure and function of the androgen receptor and its role in prostate cancer progression.

KEYWORDS: Androgen, polymorphism, androgen dependence, androgen independence, prostate cancer, androgen blockade, antiandrogen, coactivators, corepressors.

1. THE PROSTATE – LOCATION AND DESCRIPTION

The prostate is a secretory gland that surrounds the urethra close to the base of the bladder (Figure 1). The average size of the gland is 3x4x2cm and it can be divided into anatomically and histologically distinct zones: a peripheral zone that represents approximately 70% of the glandular bulk, a central zone that accounts for about 20%, a transitional zone that accounts for 5% and a non-glandular anterior fibromuscular zone of stroma (Figure 1) (11, 100). The stroma, which is continuous with the capsule that encases the prostate, consists of collagen, elastin and smooth muscle. Upon ejaculation, contraction of the muscle forces prostatic secretions into the urethra. Secretions from the male accessory sex glands have been shown to be important in events such as semen coagulation and liquefaction, coating and decoating of spermatozoa and conditioning of the urethral surface (1).

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Growth of the prostate is dependent on androgens, particularly dihydrotestosterone (25). This drives growth of the prostate during development and prostate function on maturity. In PCa and benign prostatic hyperplasia, androgen-dependent growth resumes. The actions of androgens are mediated by the androgen receptor (AR), a member of the nuclear receptor (NR) superfamily and a ligand-activated transcription factor. This binds to response elements in the regulatory sequence of target genes, ultimately leading to prostate growth and differentiation.

2. THE ANDROGEN RECEPTOR

The human AR gene is situated on the X chromosome at Xq11-12 and consists of 8 exons spanning 90kb (12, 46, 77, 88, 93), which code for a protein of approximately 110kDa in molecular weight. Like other NRs, the AR has a modular structure (reviewed in 121) – see Figure 2. The N-terminal domain is highly variable in sequence and size across the NR family, ranging from less than 50 to more than 500 amino acids. The most highly conserved region between NRs is the central DNA binding domain (DBD). The carboxyl-terminal ligand binding domain (LBD) is moderately conserved (121). Exon 1 encodes the N-terminal domain, which contains activation

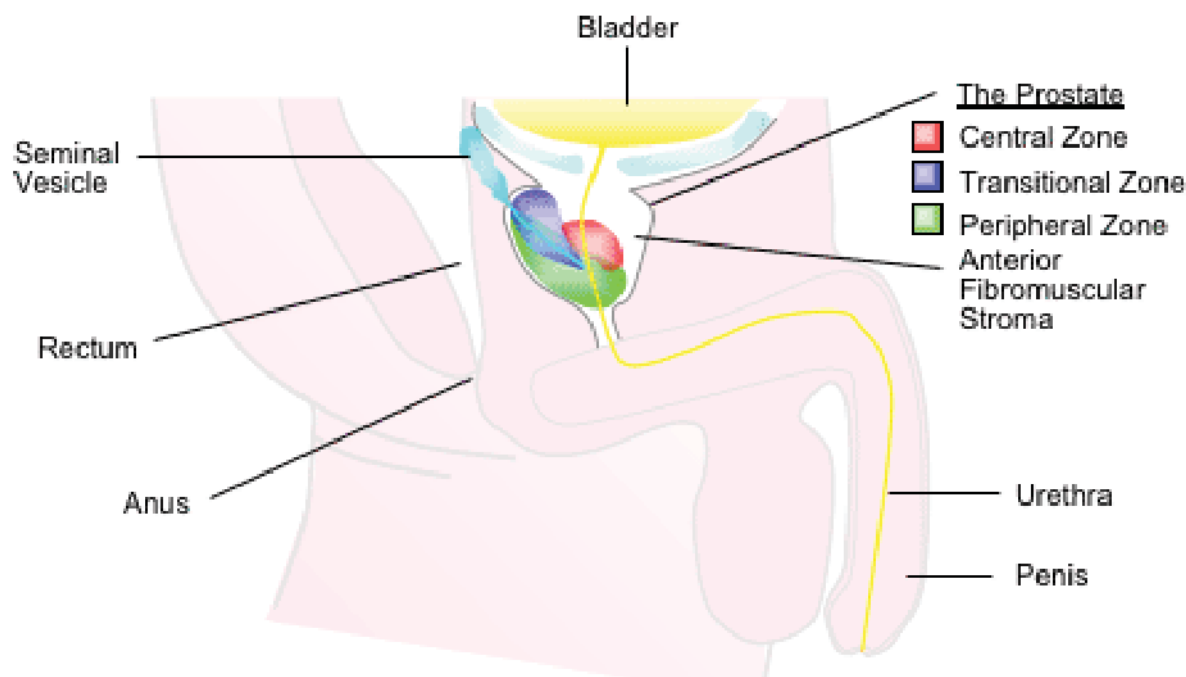


Figure 1: Location and zones of the prostate gland.

function-1 (AF-1) and the entire 5' untranslated region (UTR) (Figure 2). Exons 2 and 3 encode the DBD, whilst exons 4-8 code for the LBD, which contains the second (ligand dependent) activation function (AF-2) (72).

2.1. DNA binding domain

Although the DBDs of NRs share high amino-acid sequence homology, between 40-95%, the difference between the amino-acids that contact the DNA confer the specificity of receptor binding to target gene response elements (158). The DBD of the AR spans amino acids 559-624, a region that includes 9 conserved cysteine residues. Eight of these cysteines form two tetrahedral conformations, each around a single zinc atom (Figure 3). This creates two zinc finger-like modules, organised into three α -helices, through which NRs specifically interact with DNA (17, 56, 89, 131). Crystallographic studies of the glucocorticoid receptor have revealed that the first zinc module of the DBD, consisting of the first α -helix and containing the P-box (corresponding to Gly⁵⁷⁷, Ser⁵⁷⁸, Cys⁵⁷⁹, Lys⁵⁸⁰ and Val⁵⁸¹ of the AR) (Figure 3), contacts the major groove of DNA. Studies using hybrid receptors have demonstrated that this region is particularly important in response element specificity (31, 90, 148). The second zinc module, which comprises of α -helices 2 and 3, stabilizes DNA binding through its interaction with the sugar-phosphate backbone of the adjacent sequence (19). In particular amino acids Ala596, Ser597, Lys598, Asn599 and Asp600, collectively known as the D-box, are crucial to this interaction (30). Recently, the AR DBD has been solved and has revealed that the AR binds to DNA in a similar manner as that found for the glucocorticoid receptor (131).

2.2. Activation function-2 and the ligand binding domain

The AR LBD contains the ligand dependent AF-2 and is functionally complex, containing regions important for heat shock protein (HSP) association (114, 152), dimerization (106), cofactor binding (65),

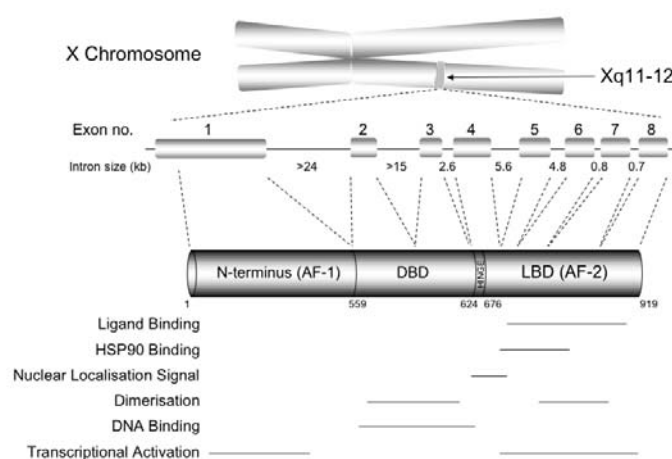


Figure 2: Modular structure and domain functions of the androgen receptor.

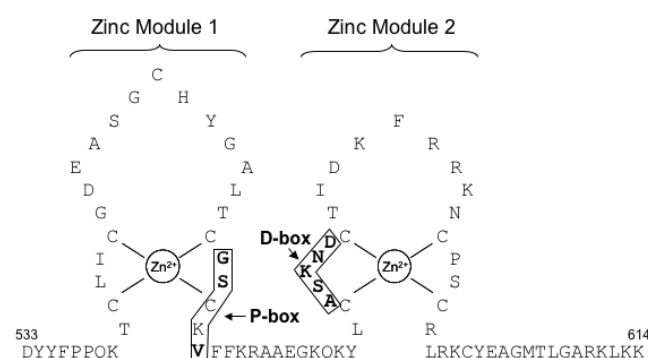


Figure 3: The Zinc Modules of the androgen receptor DNA binding domain. The figure gives the amino acid sequence for the AR DBD and highlights the P-box and D-box.

cellular localisation and hormone binding (136). The crystal structures of the LBD of several nuclear receptors, including the AR (94, 127), have been solved and reveal that the structure of NR LBDs is highly conserved, although the exact dimensions of the pocket vary to suit the ligand (10, 13, 41, 78, 113, 116, 122, 139, 149, 159). These crystal structures have revealed a triple-layered anti-parallel α -helical sandwich fold composed of 1 β -sheet (S1/S2) and 11-12 α -helices (H1-H12) (48). The hydrophobic ligand-binding pocket is principally composed of residues from H3, H5, loop L7-8, H8, H11, H12 and S1/S2 (Figure 4). Following the solving of the crystal structure of NR LBDs in the presence and absence of ligand, it has become evident that upon ligand binding, helix 12 realigns and acts as a lid to the ligand binding pocket (Figure 4) (110, 161). This relocalisation of helix 12 creates a coactivator interaction surface consisting of helices 3, 4, 5 and 12 that is important in transcriptional activation (91).

2.3. Activation function-1

The AR AF-1 has been shown to be the predominant activation function since deletion of AF-2 and the LBD results in a constitutively active receptor that has similar activity to the full-length AR in the presence of ligand (37, 164). This is in contrast to most other NRs, in which AF-2 is the predominant activation function. Activation function-2 of the oestrogen receptor for example, has significantly higher activity than AF-1 (85). Extending the sequence of the isolated AR AF-1, to include parts of the LBD, reduces its activity in the absence of hormone, suggesting that the LBD can inhibit the ligand-independent activity of AF-1, perhaps via direct interactions or recruitment of co-factors (164). The structure of AF-1 is relatively flexible, but upon interaction with cofactors, AF-1 adopts a more folded structure which enhances subsequent protein-protein interactions (98).

3. THE ANDROGEN RECEPTOR PATHWAY

3.1. Nuclear Translocation

The unliganded AR is normally diffuse throughout the cytoplasm (147), associated with a large heterocomplex having a molecular weight of 250-300 kDa. Many of the component proteins of this complex have been identified, for example the heat shock proteins HSP70 and HSP90 (152). After binding ligand, the AR undergoes a conformational change which leads to dissociation of the HSPs (152). This unmasks the nuclear localisation signal (NLS), promoting receptor translocation to the nucleus (48, 137, 164). The NLS of the AR is a bipartite sequence, consisting of 2 basic motifs separated by 10 amino acids, that overlaps the DBD and hinge region (between amino-acids 617-633, see Figure 2) (164). Following nuclear translocation, the AR has been found to localise into nuclear foci, along with other factors important in receptor activity (74, 120). Interestingly only AR bound to either pure or partial agonists localise to such foci. Although complete antagonists can promote nuclear translocation, in the presence of such ligands the receptor has a more diffuse nuclear localisation (147).

3.2. DNA binding

Through the use of *in vitro* selection procedures, the AR has been found to bind strongly to an inverted imperfect repeat of a 5'-TGT-TCT-3' half site (referred to as the core recognition sequence) separated by 3 base pairs (6, 18, 57). Androgen response elements (AREs) similar to this consensus sequence have been found located up- or down-stream of the transcriptional start site of many androgen responsive genes (22, 33, 35, 55, 84, 105, 119, 154). These response elements are promiscuous, since the glucocorticoid, mineralocorticoid and progesterone receptors also bind to them (57, 125, 146). It would therefore seem that androgen dependent genes may be activated by a number of NRs, especially since, in most target organs, the glucocorticoid receptor is also present and sometimes in greater abundance (23).

The mechanisms of steroid specificity *in vivo* are not completely understood, but receptor distribution and hormone metabolism are likely to be significant (24, 44). Other mechanisms of selectivity that have been proposed include differential recruitment of cofactors (145) and selectivity due to differences in the type of response element present (153). Recently a second class of AREs, that appear to be highly selective for the AR, have been identified in the promoter region of androgen responsive genes, such as the rat probasin, mouse liver-specific sex limited protein and human secretory component (SC) genes (5, 23, 54, 87, 107, 111, 117, 154). This class of AREs, for which the other steroid receptors have low affinity (128), consists of 2 hexameric half-sites arranged as direct repeats separated by 3bp of spacer, with the half site repeating on the same strand (131).

The crystal structure of the glucocorticoid and oestrogen receptor DBDs in complex with palindromic response elements has revealed that the receptors dimerise in a head-to-head conformation (89, 129). It was proposed that on direct repeats, the AR would form a head-to-tail dimer (23) - as seen for the Vitamin D Receptor (VDR) DBD bound to a direct repeat response element (130, 131), similar to those found to be specific for the AR. Resolution of the crystal structure of the AR DBD in complex with DNA has disproved this hypothesis, however, revealing that ARs dimerise in a head-to-head

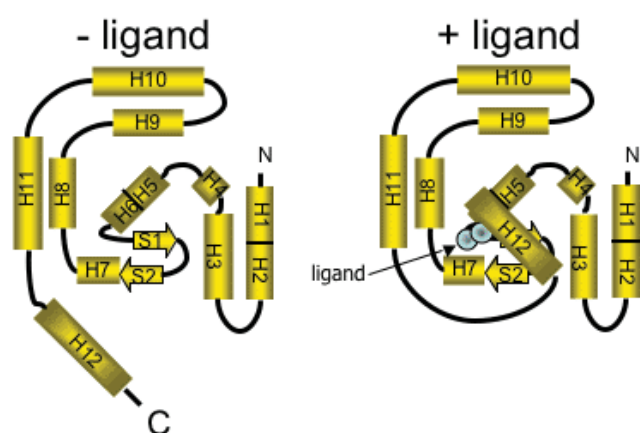


Figure 4: Canonical representation of the androgen receptor ligand binding domain in the presence and absence of ligand. α -helices labelled H1-H12 and β -sheets labelled S1 and S2 (modified from 76).

formation on both direct and inverted response elements (131).

3.3. N- and C-terminal interaction

The conformation change induced by ligand binding promotes an interaction of the N- and C-termini of the AR. There is evidence for this agonist induced interaction in a number of nuclear receptors (for example oestrogen receptor γ , peroxisome proliferators activated receptor α , and the progesterone receptor) but it appears to be most important and has been best characterised for the AR (7, 14, 69, 76, 82, 83, 101, 133, 138, 142, 143). The interaction between the 2 termini has been shown to increase receptor stability, to reduce ligand-off rate and to be necessary for maximal activation (58, 61, 82). A mutation found in the CAG repeat region of the AR N-terminus (interruption of the CAG repeat by 2 non-consecutive leucine residues) disrupts the inter-domain communication and reduces AR protein levels, potentially due to loss of stability (15). Paradoxically, this mutant had greater transcriptional activity than the WT AR, revealing that the N-/C-terminal interaction is not necessary for maximal activation in certain circumstances. Further, the requirement of the N-/C-terminal interaction for maximal activity appears to be dependent upon promoter context (16, 62).

Two motifs in the AR N-terminal domain have been identified as important for the interaction between the N- and C-termini: ²³FQNLF²⁷ and ⁴³³WHTLF⁴³⁷ (61). The FQNLF motif is the predominant one and is similar to the LxxLL receptor interacting motifs found in coactivators. It interacts with AF-2 with a higher affinity than the LxxLL motifs, suggesting that the N-/C-terminal interaction may be inhibitory to LxxLL-dependent recruitment of coactivators to AF-2 (58). It has been postulated that there is equilibrium between the intra-receptor interaction and the interaction of coactivators with AF-2. Over-expression of coactivators (predicted to be a potential mechanism of hormone therapy failure, see section 5.3.2) may shift the equilibrium in the favour of coactivator LxxLL-dependent interactions, which has been hypothesised to lead to higher transcriptional activity of the receptor (63).

3.4. DNA remodelling

Nucleosome positioning on DNA has direct effects upon the accessibility of transcription factors to target gene regulatory sequences, with the protein-DNA interactions present in chromatin restricting accessibility of such factors. Thus, for the transcription of a target gene, transcription factors need to recruit proteins that can modify chromatin structure. Two mechanisms by which this can be achieved are post-translational modification of histones and the action of ATP-dependent chromatin remodelling complexes (102). Histones are composed of a structured domain and an unstructured N-terminal 'tail' of 25-40 residues (reviewed in 51). These tails protrude from the nucleosome into the surrounding space and are sites for post-translational modifications (51). The best studied modification is acetylation of lysine residues and the effect that this has upon chromatin structure (reviewed in 73, 109). In general, hyperacetylation of histones by HATs (Histone Acetyltransferases) correlates with transcriptional activation, since acetylation of the histone tails destabilizes the contacts between the histones and DNA allowing access for DNA-binding factors (123, 160). Opposing the action of HATs are HDACs (Histone Deacetylases), which reduce the acetylation of histone tails and therefore induce chromatin compaction.

3.5. Coactivators

Coactivators are proteins that do not bind DNA directly, but increase the transcription of genes by interacting with transcription factors. The hormonal regulation of target genes is dependent upon recruitment to receptors of active transcription initiation complexes containing such factors (99, 144). Coactivators either have, or recruit proteins that have, HAT activity and act as adapter molecules between nuclear receptors and the basal transcription machinery (65, 99). This promotes the formation of stable pre-initiation complexes that subsequently promote RNA synthesis (75, 124).

Many coactivators that interact with the AR have been described (reviewed in 65). Several of these can be divided into three different classes: the p160 family; CBP/p300; the PIAS (Protein Inhibitor of Activated STAT protein 1) family; and the SWI/SNF/BRG and TRAP/DRIP (T₃R Receptor Associated Protein/Vitamin Receptor D Interacting Protein) complexes (47). Probably the best characterised are the p160 family of coactivators, consisting of 3 proteins: SRC-1/NCOA1 (Steroid Receptor Coactivator-1 / Nuclear Receptor Coactivator 1), SRC-2/NCOA2/TIF-2/GRIP-1 (Transcriptional Intermediary Factor 2 / Glucocorticoid Receptor Interacting Protein-1) and SRC-3/NCOA3/AIB1/pCIP/RAC-3 (Amplified In Breast Cancer-1 Protein / CBP-Interacting Protein / Receptor-Associated Coactivator 3) (21).

Many coactivators have been shown to interact with NRs via LxxLL motifs (where L is leucine and x is any other amino acid), which form an amphipathic α -helix that binds to the ligand-dependent hydrophobic coactivator interaction surface (the AF-2 core) in the nuclear receptor LBD (32, 36, 64, 108, 135). In contrast to other steroid receptors, this interaction appears to be relatively weak for the AR (60). The AR LBD preferentially interacts with FxxLF-like (where F is phenylalanine) motifs (59, 68). Computer modelling of the 3D structure of the AR LBD complexed with an FxxLF motif revealed that the bulky F residues are buried in a deep groove and the conformation of this groove can adjust to allow binding of both FxxLF and LxxLL-like motifs (68). Phenylalanine-rich motifs have been identified in the N-terminus of the AR (see section 4.3) and in some coactivators, for example Four-and-a-Half Lim-Only Protein 2 (FHL2) (68). Interestingly, this coactivator appears to be AR-specific providing further evidence that the strong interaction with FxxLF-like motifs is unique to the AR (103). It should be noted, however, that some cofactors are also able to interact with other regions of the receptor in an LxxLL/FxxLF motif-independent manner. Steroid Receptor Coactivator 1, for example, not only interacts with H12 of the AR through the LxxLL motifs, but also interacts more strongly with AF-1 via a glutamine-rich region (9).

3.6. Corepressors

Corepressors are proteins that do not bind DNA directly, but reduce the transcription of genes by binding to transcription factors (4). The first corepressors identified were N-CoR and SMRT (Nuclear Receptor Corepressor and Silencing Mediator for Retinoid and Thyroid Hormone Receptor), which have been shown to interact with certain constitutively nuclear NRs (for example the thyroid receptor, retinoic acid receptor and vitamin D receptor) in the absence of cognate hormone and, via the recruitment of HDACs, promote gene silencing (reviewed in 115). The interaction between these corepressors and the unliganded receptors is via a LxxI/HxxxI/L motif (where L is leucine, x is any amino acid, I is isoleucine and H is histidine)

- an extended version of the coactivator interaction motif (112). This repressor motif interacts with the same region of the receptor as coactivators (104).

Not all receptors bind corepressors in the absence of ligand. Steroid receptors appear to preferentially interact with such factors in the presence of antagonists (4). The progesterone receptor for example, in the absence of ligand or the presence of progesterone, interacts weakly with NCoR (163). In the presence of an anti-progestin (Mifepristone) however, a much stronger interaction between the progesterone receptor and NCoR is evident. Chromatin Immunoprecipitation (ChIP) studies of the PSA (prostate specific antigen) promoter also suggest that the AR interacts with NCoR and SMRT (although a direct interaction has not been shown). Neither of these repressors are present at AREs when agonist induced AR is present (132). In the presence of a pure antagonist (bicalutamide) the AR is still found to bind AREs, but NCoR and SMRT are also present. The antagonist-induced repression by these cofactors therefore appears to be via the active recruitment of a repressive complex to the promoter region.

4. PROSTATE CANCER

4.1. Prostate cancer epidemiology

Each year in the UK over 134,000 men are diagnosed with cancer, with every man having a 1 in 3 chance of being diagnosed with a form of cancer at some point in their lifetime (28). The highest incidence rate is for Prostate Cancer (PCa), with approximately 27,000 men diagnosed per year, followed by lung (23,000 men diagnosed) and colorectal cancer (19,000 men diagnosed). Although the highest cancer-related death rate is due to lung cancer (20,200 deaths in 2002), its incidence is decreasing due to the reduction in people smoking. For this reason and since the average age of onset of PCa is falling (67), it is likely that deaths caused by PCa (presently 9,900 per year) will eventually overtake lung cancer as the leading cause of cancer-related death in Western men. Unlike most other types of cancer, not every prostate tumour constitutes a serious risk of morbidity to the affected person. Since PCa is a disease of elderly men and often slow in progression, many die with PCa rather than from it.

Survival of patients diagnosed with organ-confined PCa is very high (5 year survival >99%). Unfortunately, a proportion of PCa foci grow rapidly and metastasise, potentially via perineural invasion (155) to the lymph nodes and subsequently to bone, often leading to death. The 5 year survival rate for patients with distant metastases is 34% (71). Improvements in detection and treatment of PCa, however, has led to an overall increase in 5 year survival rates – from 31% between 1971-5 to 65% between 1996-9 (28).

4.2. Prostate cancer treatment

The management of PCa is a controversial issue (reviewed in 95). This is due to the many different treatments available, side-effects associated with these treatments and the fact that presently there is no way of distinguishing which patients will have latent disease from those that will develop aggressive forms of the disease. The controversy has been further compounded by the lack of randomised prospective studies, with analysis based entirely upon single institution retrospective analysis. There is, however, growing evidence that patients with organ-confined disease tend to benefit from treatment and that,

of the available treatments, radical prostatectomy offers the highest likelihood of long-term disease free survival (134). Other treatments for organ-confined PCa include external-beam radiotherapy, cryosurgical ablation, high-intensity focused ultrasound and a policy of watchful waiting (monitoring patients for disease progression before initiation of treatment). Unfortunately approximately 75% of patients present with non-organ confined disease (43). Since the growth of almost all cases of PCa is dependent upon the AR and its pathway (42), total androgen blockade (a combination of chemical castration and antiandrogens) is used to reduce this signalling axis.

Androgen blockade induces cell cycle arrest and cell death in prostatic adenocarcinoma, and is highly effective since more than 80% of patients respond to the treatment (34, 80). Chemical castration is achieved with the use of Luteinizing Hormone Releasing Hormone (LHRH) analogues. Normally LHRH, via receptors in the pituitary gland, stimulates the release from the pituitary gland of Luteinizing Hormone (LH), which in turn increases the production of testosterone by the testes. Analogues of LHRH over-stimulate and desensitise the receptors, resulting in a decrease in LH and a subsequent reduction in testicular testosterone synthesis. Although this therapy can reduce circulating testosterone by approximately 95%, substantial levels of adrenally produced androgen precursors remain in the blood. These can be effectively converted to dihydrotestosterone (DHT) in the prostate, so local levels of DHT are only reduced by 60% (80, 81). Due to these residual levels of androgens, antiandrogens (compounds that bind to the AR and hold it in an inactive state) are also administered.

Several theories have been proposed to explain how anti-androgens function – for example blocking the receptor from interacting with DNA or from inhibiting the recruitment of the necessary factors for transcription initiation (see Figure 5). Whitaker et al. found that antiandrogens appear to work via different mechanisms (157). Treatment of cells with cyproterone acetate resulted in AR association with cytoplasmic membranes and irreversible retention within the cytoplasm. Bicalutamide, in contrast, was found to target the AR to the nuclear matrix (157). In another study, chromatin immunoprecipitation (ChIP) studies revealed that bicalutamide bound ART877A (a mutant form of AR that has loss of ligand specificity but for which bicalutamide is still antagonistic) can still bind to the promoters of target genes. Instead of interacting with cofactors that are necessary for transcription initiation, however, the receptor was found to promote the recruitment of corepressors (such as NCoR and SMRT) to the response element (132).

Androgen blockade is not curative and the PSA response (reduction in circulating PSA levels used as a measure of response to therapy) only lasts for a median duration of one year (95). At symptomatic relapse, on average 2 years after the PSA relapse, the median duration of survival is 6-8 months (49). When the tumour relapses and is able to grow in the androgen depleted environment it is referred to as Androgen Independent or Hormone Refractory. Since few treatment options exist for this stage of the disease it is hoped that increased understanding of the transition from androgen dependence to androgen independence may offer novel targets for the treatment of advanced PCa.

4.3. Hormone independence of prostate cancer

Isaacs proposed the clonal expansion theory to explain androgen independent growth of PCa, which states that androgen independent tumour cells may co-exist with androgen dependent cells at the time

of initial treatment and become predominant by selection during hormonal therapy (70). The majority of androgen independent tumours still express the AR (86, 126) and so it is possible that selection favours mechanisms by which the AR can remain active even in the androgen-depleted environment. A number of hypotheses have been proposed to explain such androgen independent growth:

4.3.1. Androgen receptor over-expression

It has been proposed that PCa can circumvent androgen ablation by an increase in sensitivity to low residual androgen levels. Androgen receptor amplification could have such an effect, and approximately 30% of androgen independent tumours have increased AR levels (156). In animal models it has been shown that increased AR ex-

pression can also be accompanied by increased receptor stability and enhanced nuclear localization (53). Cell lines (CWR-R1 and LNCaP C4-2) harboring these abnormalities require a DHT concentration, for growth stimulation, 4 orders of magnitude lower than that required for androgen-sensitive LNCaP cells (a prostate cancer cell line derived from a lymph node containing metastatic lesions) (66).

Studies of xenograft models of several androgen sensitive PCa cell lines and their paired androgen independent lines (created by serial passage in castrated male mice) found that the only consistent change was up-regulation of the AR (20). Stable over-expression of the WT AR in LNCaP cells resulted in bicalutamide becoming agonistic for target gene expression and growth. The exact mechanism by which over-expression of the AR can transform antagonists into agonists is not completely understood. One hypothesis is that levels of corepressors are limiting and so if the AR is in excess the antagonist bound receptor may inappropriately bind coactivators (Figure 6). Evidence for this comes from ChIP experiments performed on the promoters of target genes (PSA and kallikrein-2) that demonstrate that in the AR over-expressing LNCaP cell line, the balance of cofactors at AREs shifts from corepressors to coactivators in the presence of bicalutamide (20).

4.3.2. Alterations in cofactor levels or function

There is conflicting evidence as to whether or not changes in cofactor expression could play a role in therapy failure. As discussed above, the microarray study of isogenic PCa xenograft models performed by Chen *et al.*, found that the only gene to be consistently associated with the development of resistance to antiandrogen therapy, was the AR (20). In contrast to this study, Gregory and colleagues found that SRC1 and TIF2 were over-expressed in recurrent PCa (52). TIF2, for example, was found to be expressed in 6 out of 8 androgen independent tumours but was not detectable in androgen dependent samples. Such up-regulation of positive cofactors is another mechanism by which the sensitivity of the AR pathway could be increased. Up-regulation of these two coactivators was found to increase AR activity in the presence of DHT, testosterone and the less potent adrenal androgens (52). Down-regulation of corepressors or inhibition of their function could also increase AR activity. The proinflammatory cytokine Interleukin-1 β (IL-1 β) has been demonstrated to induce nuclear export of the NCoR complex hence reducing their effective concentration (3), and treatment of LNCaP cells with IL-1 β can convert antagonists (bicalutamide and cyproterone acetate) into agonists. As mentioned above, ChIP analysis of the PSA and kallikrein-2 promoters has demonstrated that repressors are present at the promoter region when cells are treated with an antiandrogen (20). When cells were treated with a combination of antiandrogen and IL-1 β , however, coactivators are instead recruited to the promoter region (2) suggesting that the nuclear export of the NCoR complex leaves the AR free to bind coactivators. The finding that macrophages can infiltrate prostate tumours suggests that this effect could occur *in vivo* (165).

4.3.3. Ligand-independent receptor activation

Hormone receptors activated by ligand-independent mechanisms have been referred to as 'outlaw' receptors (42). Some growth factors, such as IGF-1, KGF and EGF can activate the unliganded AR in certain conditions, leading to transcription of target genes (29). IGF-1, for example, was able to induce a 5 fold increase in expression of PSA in LNCaP cells (29). This activity was completely blocked by bicalutamide, suggesting that the action of IGF-1 on PSA was via the AR. Culig *et al.* have also found that such growth factors are

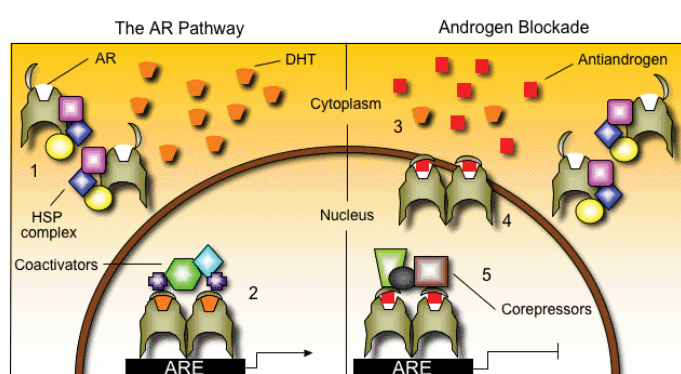


Figure 5: Androgen blockade inhibits the AR signalling axis. In the absence of ligand, the AR exists in the cytoplasm in a complex with heat shock proteins (HSP) that hold the receptor in a state ready to bind ligand (1). Upon ligand (DHT) binding, the AR translocates to the nucleus, forms homodimers, binds to androgen response elements (AREs) in or near the promoters of target genes and recruits coactivators and the basal transcription machinery (2). Although LHRH analogues successfully reduce the production of androgens in the testes they do not affect adrenal production of androgens, hence residual levels of androgens exist in the prostate (3). For this reason antiandrogens are administered with the aim of holding the receptor in an inactive state. Several hypotheses have been proposed to explain how antiandrogens inhibit AR function, for example targeting the receptor to sub-compartments of the nucleus or cytoplasm where it is unable to bind DNA (4). Evidence also exists that the antagonist bound AR can interact with DNA, but is unable to recruit the necessary factors to induce transcription and instead corepressors are found to be present at the response element (5).

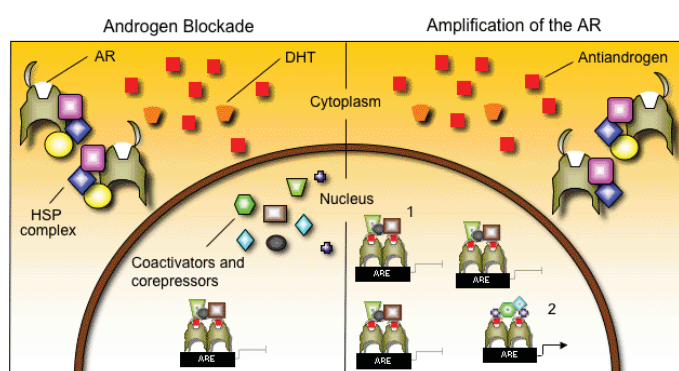


Figure 6: Schematic to show the potential mechanism by which amplification of the AR may drive hormone independent growth. (1) The antagonist bound AR, when DNA bound, is associated with a corepressor complex. (2) Amplification of the AR could result in a shortage of corepressors and hence the AR could inappropriately interact with coactivators.

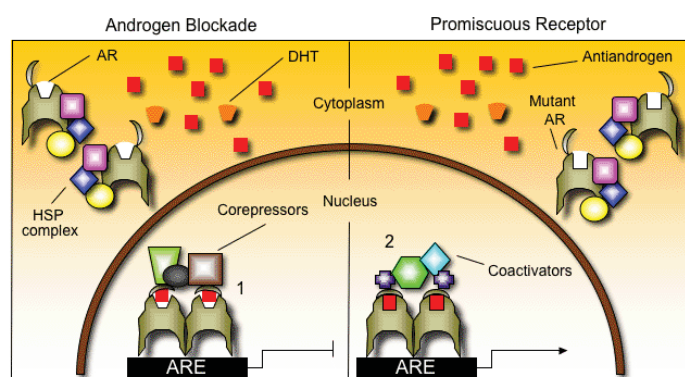


Figure 7: Schematic to show the potential mechanism by which mutations of the AR, that cause promiscuity, may drive hormone independent growth. (1) The antagonist bound AR, when DNA bound, is associated with a corepressor complex. (2) Mutations of the receptor appear to allow the alternative ligands (for example anti-androgens) to fit into the ligand binding pocket and allow an active conformation.

over-expressed in some cases of PCa (29). Other studies have found that other putative 'outlaw' pathways exist, including up-regulation of the receptor tyrosine kinase HER-2/neu protein, which has been shown to be frequently over-expressed in xenograft derived androgen independent sub-lines (27). Following this finding, the group over-expressed HER-2/neu in an androgen dependent cell line and found that it allowed the cells to grow in an androgen independent manner. HER-2/neu can stimulate expression of AR dependent genes, but not in the absence of the AR. Evidence suggests that HER-2/neu may be activating the MAP kinase pathway, which upon phosphorylating the AR, increases transcriptional activity by promoting receptor-coactivator interactions (162). It was found, however, that the inappropriate activity induced by HER-2/neu over expression could not be blocked by treatment with bicalutamide, indicating that the activity is independent of the AR LBD (27).

4.3.4. Promiscuous activation of the androgen receptor

Many somatic mutations of the AR have been identified in cases of PCa, currently approximately 70 presently described. Many of these lie in the LBD coding sequence (50). Mutations of the AR are rare in early stages of PCa, but their incidence increases significantly after androgen ablation and subsequent transition to androgen independence, suggesting that they are important in disease progression (92, 140). One study, for example, found that out of 99 patients with early stage PCa, none had mutations in the AR coding sequence (92). Out of 38 patients with more advanced disease, however, 8 were found to have mutant forms of the receptor (92). From the relatively small number of mutants that have been presently studied at the functional level, it has emerged that many allow alternative ligands (for example other hormones and antiandrogens) to bind and activate the receptor (Figure 7), thus may provide a growth advantage by reducing ligand specificity. Cells carrying such mutations could be selected for by hormonal treatment and the tumor recur via clonal outgrowth (141).

The first AR mutation with loss of ligand specificity to be described was the T877A substitution (a threonine to alanine substitution at amino acid 877) (150). This mutant, which is present in the AR of the LNCaP PCa cell line, has been frequently found in advanced prostatic carcinomas (45, 50, 140, 141). Gaddipati *et al.* found the T877A mutation in 25% of metastatic PCa samples analysed (45) and Taplin *et al.* found the mutation in 30% of bone marrow metas-

tases (140). The effect of the T877A mutation in the LNCaP model has been well studied and has been shown to be activated not only by androgens but also by oestrogens, progestins and the anti-androgens cyproterone acetate and hydroxyflutamide (the active form of flutamide) (reviewed in 151). Bicalutamide, however, is able to block AR target gene transcription and cell growth (8).

Mutations in the LBD of the AR appear to reduce ligand specificity via different mechanisms. Crystallographic analysis of the AR LBD in complex with a synthetic androgen has revealed that threonine 877 is one of the 18 amino acids that contacts the cognate ligand (94). It has been proposed that substitution of threonine with alanine (T877A) alters the size and shape of the pocket, thus allowing alternative ligands to fit into the pocket and to induce an active conformation. Another mutation found in PCa is a substitution from histidine to tyrosine at amino acid 874 (H874Y). The side chain of amino acid H874 points away from the bound ligand and is not expected to alter the size and shape of the pocket. Instead, H874 lies in a cavity between helices 11 and 12, which is formed when helix 12 relocates after agonist binding (96). The cavity is large enough to accommodate the tyrosine aromatic ring and so the H874Y substitution is not expected to cause a steric change at this site. It has been proposed, however, that the more hydrophobic tyrosine side chain could affect the strength of interaction between helix 12 and this crevice (96). This stronger interaction could therefore enable helix 12 to relocate to the active conformation even when a ligand is bound that does not optimally fit into the pocket. This increased binding appears to have an affect upon coactivator recruitment since the H874Y mutant has been shown to have higher affinity for the p160 coactivators and also have higher activity in their presence, compared to the wild-type receptor (38).

The 'antiandrogen withdrawal affect', which is commonly seen after failure of hormone therapy, provides further evidence of the clinical importance of AR mutations. In these cases, continued treatment with the antagonist leads to symptomatic worsening, but after withdrawal of the antiandrogen, disease status improves (reviewed in 118). This may be linked to proliferation of cells carrying AR variants activated by the anti-androgen.

4.3.5. Androgen receptor-independent pathways

Although this review has concentrated upon the AR and its role in the progression of PCa to androgen independence, evidence exists to suggest that in some cases up-regulation of parallel pathways can provide a substitute survival signal that allows the cancer to bypass the reliance of growth upon the AR pathway. One such pathway is that of the anti-apoptotic proto-oncogene BCL2 (B-cell CLL/lymphoma 2). BCL2 is not normally expressed in the secretory epithelial cells of the prostate (97), but has been found in premalignant prostatic intraepithelial neoplasia (PIN) and some cases of androgen independent PCa (26).

5. CONCLUSION

PCa growth is almost always dependent upon the AR. Therapies that block this pathway are successful at reducing tumour growth, but invariably fail and the tumour progresses in the androgen-depleted environment. The work highlighted in this review suggests that the AR remains a valid therapeutic target in the majority of androgen independent cancers. Recently several novel non-pharmacological approaches to either reduce AR levels or block receptor function have been published. Expression of a 15-mer antisense oligonucle-

otide that hybridises with the N-terminus of the AR, for example, has been demonstrated to reduce AR levels in LNCaP cells (39). Xenograft models using this cell line revealed that down-regulating the AR using this approach can also reduce tumour growth *in vivo* (40). In another study, expression of a short piece of DNA (23-mer double-stranded oligonucleotide) containing an ARE was also found to reduce AR activity by acting as a decoy binding site and presumably sequestering AR (79). Understanding of the mechanisms of androgen independent growth has therefore been useful in the design of novel therapies for this stage of the disease. Presently, few treatment options exist for patients with androgen independent PCa and so continued investigation within this field is needed to find ways in which to block the progression from androgen dependence to androgen independence, or to provide new treatment options for when androgen ablation fails.

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